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## Theory of Bacterial Detoxification.

Bacterial detoxification may be defined as the neutralization of the toxic products of bacterial cells whereby the toxin is still able to stimulate the production of antibodies but is unable to exert its toxic effect on the body of the animal in which it is contained. Effective germicides and antiseptics combat the attack of bacteria but, if effective, they also circumvent antibody production. Detoxifiers neutralize the attack of bacteria without necessarily killing them, although inhibiting their physiological processes, and allowing the production of antibodies to continue. This method of treating the bacterial infections is, therefore, advantageous for it renders the body immune to reinfection by the organism detoxified.

## The Role of Surface Tension in Detoxification.

One of the better bacterial detoxifiers is sodium ricinoleate. In researches with organisms in respect to the effect of sodium ricinoleate on their toxicity it has been observed (57a) that those organisms which normally grow at a low surface tension are relatively insensitive to the action of sodium ricinoleate. On the other hand, those organisms normally accustomed to rather higher surface tensions are quite sensitive to its action. It was this curious and interesting fact which led workers to postulate that detoxification is essentially due to the reduction of the surface tension of the fluid in which the bacteria are living.

The surface tension of a liquid may be regarded as being the seat of a special force. This force, called the surface tension, acting perpendicularly to a section of the surface one centimeter in length is generally measured in dynes. Surface tension exists at the interface between liquid and air. A surface tension also exists at the

boundary of two immiscible liquids, between a liquid and a solid, and indeed, wherever a free surface is formed.

Larson and his associates have found ample evidence that surface tension affects bacterial growth. They caused (52) surface growing bacteria to grow throughout the medium by decreasing the surface tension from 59 dynes to 40 dynes. Organisms normally found in the intestine grow abundantly on media of low surface tension. This is to be expected, as the contents of the intestinal tract have a low surface tension due to the presence of the bile salts, soaps and other surface tension depressants. Governed by well-known physical laws, the surface tension depressants in culture media concentrate in the surface layers of the media (55). This explains why the colon-typhoid-cholera group selects the surface zone for growth. It is for this reason that pellicle formers grow as they do, at the top of the broth medium where the surface tension depressants are concentrated. If the interfacial tension between liquid and bacterial cells is sufficiently depressed, the organisms grow throughout the medium and even at the bottom. On the other hand, by increasing the surface tension, non-pellicle formers may be made to form pellicles.

Tubercle bacilli, when grown for ten days at 44 dynes, lost their pathogenicity to guinea pigs (62). In explanation of this Larson suggested that when tubercle bacilli are growing diffusely throughout the medium they are "wetted" and that when they are introduced into the animal body they are the more easily penetrated by antibodies or bactericidal substances present, and destroyed. He found that when equal amounts of tubercular sputum and a 2% solution of sodium ricinoleate were mixed and allowed to stand several hours and portions of this injected into guinea pigs, tuberculosis did not appear. All of the controls died of tuberculosis.

Bacteria, like colloid particles, carry electrical charges and wander in an electrical field to one pole or the other. The surfaces of such particles are, therefore, the seat of free electrical energy. They must also be the seat of free mechanical energy arising from the surface tension of the field in which they are suspended. If oppositely charged particles or ions can establish an equilibrium by reducing the amount of free electrical energy by moving to such surfaces, they will do so in obedience to the second law of thermodynamics. If these particles or ions have the power of reducing the amount of free mechanical surface energy by virtue of an ability to lower surface tension, they would also come under the influence of mechanical, as well as electrical, ad-

sorption forces. A detoxifier which does not ionize extensively is not greatly influenced by electrical charges and is to a much greater extent dependent on mechanical adsorption, and therefore, upon its own power of reducing surface tension. Chemicals, in other words, which are not highly ionized, depend largely upon their ability to lower surface tension in order to be adsorbed. It seems agreed that the sodium ricinoleate is adsorbed onto the surfaces of the toxin molecule, "imprisoning" it so that it is not free to react with the tissues. This imprisonment is not permanent, for this would influence its antigenic properties. Rather, the imprisonment lasts for a time long enough to allow the production of antibodies. When the toxin molecule is then freed it is attacked by the antibodies and disease symptoms are prevented, while antibody production presumably continues, thus building up immunity.

Wolf's point (124) that "in the contest for the surface layer those substances which are powerful in reducing the surface tension tend to remove that which is less efficient" may be important and aid in solving the problem. Substances like sodium ricinoleate which lower the surface tension thereby expel the nutrient substances of less surface tension depressing power from the surface of the liquid. The surface then no longer contains rich nutrient substances but has a high concentration of ricinoleate which may be indirectly toxic to bacteria at or near the interfaces.

To sum up, therefore, it may be said that the mechanism of the action of sodium ricinoleate in detoxifying bacteria is as yet only theoretically explainable. It appears that surface tension is not the sole cause of its action, although it seems to be a contributory factor. The other factor of importance is a chemical one having to do with molecular structure.

## Historical.

Dr. H. Vincent of the Pasteur Institute has perhaps the greatest claim to being the "father of detoxification"—or, as he calls it, "cryptotoxication." It was he who in 1907 found that the bile constituents neutralized tetanus toxin and that bile thus has cryptotoxic properties (112). In the same year (113) he said "The antitoxic properties of soaps in very weak concentration appear worth mentioning." He found that cholesterol, lecithin and the bile salts have these properties. In 1909 he stated (115): "The most active (detoxifiers) are soaps, cholesterol and sodium glycocholate." Scattered

papers appeared on detoxification between 1909 and 1920. It was at about this time that Larson and others discovered the detoxifying powers of sodium ricinoleate and in 1923, Larson and Montank (62) reported the neutralization of the tubercle bacillus by a 2% sodium ricinoleate solution. Sodium ricinoleate was made still more pure and its detoxifying powers were greatly increased. Many papers on detoxification with ricinoleate have appeared in the journals of this country. These have been summarized in the tables.

## Known Bacterial Detoxifiers.

Ultra-violet light (69, 79, 123), sunlight (32), light and eosin (68), heat (46), heat and formaldehyde (31, 70) are physical agents which detoxify bacteria.

TABLE I.

Bacterial Detoxification by Physical Agencies.

| Bacterial toxin | Agent | Authors | Reference |
|---|---|---|---|
| Diphtheria | Ultra-violet light | Lowenstein | 69 |
| General | Formaldehyde and heat | Eisler and Lowenstein | 31 |
| | Formaldehyde and heat | Lowenstein | 70 |
| Tetanus | Sunlight (15 hours) | Fernie and Pernosie | 32 |
| | Ultra-violet light | Megrail and Welch | 79 |
| | Ultra-violet light | Welch | 123 |
| | Heat (68° C. for 5 min.) | Kitasato | 46 |
| | Eosin and light | Lowenstein | 68 |

TABLE II.

Bacterial Detoxification by Inorganic Agencies.

| Bacterial toxin | Agent | Authors | Reference |
|---|---|---|---|
| Diphtheria | Aluminum sulphate | Glenny | 38 |
| | Ammonium thiosulphate | Schumacher | 99 |
| | Copper | Laubenheimer | 65 |
| | Copper chloride | Laubenheimer | 65 |
| | Gold salts | Behring | 7 |
| | | Osman | 89 |

TABLE II—cont.

| Bacterial toxin | Agent | Authors | Reference |
|---|---|---|---|
| | Iodine trichloride | Behring | 7 |
| | Mineral water | Billard | 10 |
| | Potassium aluminum sulphate | Glenny | 38 |
| General | Chlorine | Moussu and Goupil | 82 |
| | Ozone | Nelis | 84 |
| | Sodium fluoride | Berthelot and Ramon | 8 |
| | Sodium perborate | Berthelot and Ramon | 8 |
| Poliomyelitis | Colloidal aluminum hydroxide | Rhoades | 93 |
| Tetanus | Copper | Laubenheimer | 65 |
| | Copper chloride | Laubenheimer | 65 |
| | Mineral water | Billard and Courtoil | 11 |
| | Radium emanation | Ferroux and Mutermilch | 34 |
| Typhus | Carbon disulphide | Lowenstein | 70 |
| | Mineral water | Ferreyrolles | 33 |

TABLE III.

BACTERIAL DETOXIFICATION BY ORGANIC AGENCIES (EXCEPTING SOAPS).

| Bacterial toxin | Agent | Authors | Reference |
|---|---|---|---|
| Botulinus | Cholesterol | Suranyi | 102 |
| Colon bacillus | Halogen compounds of salicylic acid | Vincent | 121 |
| Diphtheria | Adrenalin | Abramov and Mishennikov | 1 |
| | Amino benzoic acid | Nishuira | 88 |
| | Cholesterol | Suranyi | 102 |
| | | Suranyi and Jarno | 103 |
| | Hydroxy-benzoic acid | Nishuira | 88 |
| | Lecithin | Almagia | 5 |
| | | Bruschettini and Calcaterra | 15 |
| | | Larson | 54 |
| | Organic acids | Roux and Yersin | 97 |
| | Phenol | Nishuira | 88 |
| | Pus | Larson | 54 |
| | Quinine | Sbarsky and Subkowa | 98 |

TABLE III—cont.

| Bacterial toxin | Agent | Authors | Reference |
|---|---|---|---|
| | Quinine bisulphate | Nelis | 84 |
| | Quinine dihydrochloride | Nelis | 84 |
| | Salicylic acid (halogen compounds) | Vincent | 121 |
| | Sodium benzoate | Nishuira | 88 |
| | Sodium salicylate | Birkhaug | 12 |
| | | Nishuira | 88 |
| | Triphenylmethane series | Coplans and Green | 21 |
| General | Acetic acid | Berthelot and Ramon | 8 |
| | Acetone | Douglas and Fleming | 27 |
| | Antipyrene | Vincent | 117, 118 |
| | Chloramine | Berthelot and Ramon | 8 |
| | Cholesterol | Suranyi | 102, 103 |
| | Formic acid | Berthelot and Ramon | 8 |
| | Pyramidon | Vincent | 117, 118 |
| | Sodium salicylate | Vincent | 118 |
| | Sodium salts of alkyl resorcinol carboxylic acid | Bleyer | 13, 14 |
| | Urotropin | Berthelot and Ramon | 8 |
| Pneumococcus | Bile | Grixoni | 40 |
| Rabies virus | Cholesterol | Almagia | 5 |
| Tetanus | Adrenalin | Abramov and Mishennikov | 1 |
| | Amino benzoic acid | Nishuira | 88 |
| | Bile | Ninni | 87 |
| | | Roger | 95 |
| | | Vincent | 110, 113, 114, 115 |
| | Brain lipoids | Loewe | 67 |
| | Cerebral emulsion | Marie and Tiffenau | 76, 77, 105 |
| | Chloroform | Lowenstein | 70 |
| | Cholesterol | Almagia | 5 |
| | | Suranyi | 102 |
| | | Suranyi and Jarno | 103 |
| | | Vincent | 112 |
| | Epinephrine | Marie | 73, 74, 75 |
| | Ethyl benzoate | Loewe | 67 |
| | Gentian violet | Hall and Taber | 41 |

TABLE III—cont.

| Bacterial toxin | Agent | Authors | Reference |
|---|---|---|---|
| | Gynocardate | Vincent | 118 |
| | Hexylresorcinol | Coleman | 20 |
| | Hydroxy benzoic acid | Nishuira | 88 |
| | Hydroxy benzoic acid (halogenated) | Vincent and Velluz | 120a |
| | Lecithin | Almagia | 5 |
| | | Bruschettini and Calcaterra | 15 |
| | | Larson | 59 |
| | | Suranyi and Jarno | 103 |
| | | Vincent | 111, 112 |
| | Liver | Vincent | 111, 114, 115, 118 |
| | Pancreatic secretions | Vincent | 111, 114, 115, 118 |
| | Papain | Marie and Tiffenau | 76 |
| | Phenol | Nishuira | 88 |
| | Salicylic acid (halogen compounds) | Vincent | 121 |
| | Sodium benzoate | Nishuira | 88 |
| | Sodium salicylate | Birkhaug | 12 |
| | | Nishuira | 88 |
| | | Vincent | 119, 120, 121 |
| | Sodium 3, 5-diiodo-o-salicylate | Vincent and Velluz | 120a, 120b |
| | Stomach secretions | Vincent | 111, 114, 115, 118 |
| | Strychnine sulphate | Valley | 106 |
| | Tetramethyl ammonium chloride | Tiffenau and Marie | 105 |
| | Triphenylmethane series | Coplans and Green | 21 |
| | Xylene | Lowenstein | 70 |

TABLE IV.

DETOXIFICATION OF BACTERIAL TOXINS BY SOAPS (EXCEPTING SODIUM RICINOLEATE).

| Bacterial toxin | Agent | Authors | Reference |
|---|---|---|---|
| Colon bacillus | Sodium acyclic acid salts | Vincent | 121 |
| | Sodium butyrate | Vincent | 121 |
| | Sodium mucate | Vincent | 121 |
| | Sodium palmitate | Vincent | 116, 117, 118 |
| | Sodium pyruvate | Vincent | 121 |
| Diphtheria | Sodium acyclic acid salts | Vincent | 121 |
| | Sodium butyrate | Vincent | 121 |
| | Sodium mucate | Vincent | 121 |
| | Sodium oleate | Nelis | 83, 84 |
| | | Vincent | 118 |
| | Sodium palmitate | Vincent | 117 |
| | Sodium pyruvate | Vincent | 121 |
| Dysentery | Sodium palmitate | Vincent | 117 |
| General | All soaps | Davison | 22 |
| Oedematus bacillus | Sodium palmitate | Vincent | 117 |
| Para-typhoid B | Sodium palmitate | Vincent | 117 |
| Pneumococcus | Sodium cholate | Nicolle and Adil-Bey | 86 |
| | Sodium dehydrocholate | Ziegler | 125, 125a, 125b |
| Tetanus | Sodium benzoate | Vincent | 120 |
| | Sodium butyrate | Vincent | 121 |
| | Sodium glycocholate | Vincent | 112 |
| | Sodium guttate | Vincent | 115 |
| | Sodium hippurate | Vincent | 120 |
| | Sodium mucate | Vincent | 120, 121 |
| | Sodium oleate | Vincent | 117, 118 |
| | Sodium oleomargarate | Vincent | 112 |
| | Sodium palmitate | Vincent | 115 |
| | Sodium pyruvate | Vincent | 121 |
| | Sodium taurocholate | Vincent | 112 |
| | Sodium salts of acyclic acids | Vincent | 121 |
| Typhoid | Sodium palmitate | Vincent | 120 |

TABLE V.

DETOXIFICATION BY SODIUM RICINOLEATE.

| Toxin treated | Detoxifier | Concentration | Subject | Results | Authors | Ref. |
|---|---|---|---|---|---|---|
| Aminata phalloides | Sodium ricinoleate | | Guinea pig | Increased toxicity | Green, Stoesser | 39 |
| Botulinus | Castor oil soap | 100 L. D. | Guinea pig | Increased toxicity | Larson, Nelson | 64 |
| Diphtheria | Sodium ricinoleate | 1% sol. 0.125 L. | Guinea pig | Detoxified | Larson, Hancock, Eder | 60 |
| Diphtheria | Castor oil soap | 100 L. D. | Guinea pig | Detoxified | Larson, Nelson | 64 |
| Peridontal disease | Sodium ricinoleate | | Human | Detoxified | Hopkins | 44 |
| Pneumococcus | Sodium ricinoleate | 12 L. D. 1% sol. | Rabbit | Detoxified | Netter, Andre, et al. | 85 |
| Pneumococcus | Sodium ricinoleate | 0.1% sol. | Guinea pig | Detoxified | Larson, Nelson | 63 |
| Rattle snake venom | Sodium ricinoleate | 5 L. D | Rabbit | Detoxified | Carmichael | 16 |
| Ricin | Sodium ricinoleate | 2000 L. D. | Rabbit | Detoxified anti-ricin | Carmichael | 17 |
| Scarlet fever | Sodium ricinoleate | 0.5% sol. | Rabbit | Detoxified agglutinins | Larson, Nelson | 63 |
| Scarlet fever | Sodium ricinoleate | 15,000 skin test doses | Children | Positive Dicks neg. | Larson, Heunekens, Colby | 61 |
| Scarlet fever | Sodium ricinoleate | 4000 skin test doses | Adults | Detoxified | Eder | 30 |
| Scarlet fever | Sodium ricinoleate | | Children | Detoxified | Colby | 18, 19 |
| Scarlet fever | Sodium ricinoleate | 2% sol. | Guinea pig | Destroyed connect. tis. | Kozlowsky | 49 |
| Tetanus | Castor oil soap | 100 L. D. | Guinea pigs | Detoxified | Larson, Nelson | 64 |
| Tetanus | Sodium ricinoleate | 100 L. D. | Guinea pigs | Detoxified | Hartzell, Larson | 42 |
| Tubercle | Sodium ricinoleate | 2% sol. | Guinea pigs | Detoxified | Larson, Montank | 62 |
| Tubercle bacillus | Sodium ricinoleate | | Human | Relieved intestinal T.B. | Kline | 47 |
| Autointoxication | Sodium ricinoleate | 6 gr. capsule | Human | Complete relief | Morris, Dorst | 81 |
| Intestinal allergy | Sodium ricinoleate | 5 gr. capsule | Human | Relief | Morris, Dorst | 81 |
| Polimyelitis | Sodium ricinoleate | | Animal | Detoxified | McKinley, Larson | 78 |
| Abdominal toxemia | Sodium ricinoleate | 2% solution | Human | Aided recovery | Lutterloh, Stroud | 72 |
| Burns | Sodium ricinoleate | | Human | Aided healing | Lutterloh | 71 |

Aluminum sulphate (38), ammonium thiosulphate (99), carbon disulphide (70), chlorine (82), colloidal aluminum hydroxide (93), copper (65), copper chloride (65), gold salts (7, 89), iodine trichloride (7), mineral water (10, 11, 33), ozone (84), potassium aluminum sulphate (38), radium emanation (34), sodium fluoride (8), and sodium perborate (8) are some of the inorganic compounds which detoxify bacteria.

Acetic acid (8), acetone (27), adrenalin (1), amino benzoic acid (88), antipyrene (117, 118), bile (40, 87, 95, 110, 113, 114, 115), brain lipoids (67), cerebral emulsion (76, 77, 105), chloramine (8), chloroform (70), cholesterol (5, 102, 103, 112), epinephrine (73, 74, 75), ethyl benzoate (67), formic acid (8), gentian violet (41), gynocardate (118), hexylresorcinol (20), hydroxy benzoic acid (88), halogenated hydroxy benzoic acid (120a), lecithin (5, 15, 59, 103, 112), liver (111, 114, 115, 118), organic acids (97), pancreatic secretions (111, 114, 115, 118), papain (76), phenol (88), pus (54), pyramidon (117, 118), quinine (98), quinine bisulphate (84), quinine dihydrochloride (84), sodium benzoate (88), sodium salicylate (12, 88, 118), salicylic acid halogen compounds (121), sodium salts of alkyl resorcinol carboxylic acid (13, 14), sodium 3–5, diiodo-o-salicylate (120a, 120b), stomach secretions (111, 114, 115, 118), strychnine sulphate (106), tetramethyl-ammonium chloride (105), triphenylmethane series (12), urotropin (8) and xylene (70) are organic compounds which are bacterial detoxifiers.

Sodium ricinoleate (16, 17, 18, 19, 30, 39, 42, 44, 47, 49, 60, 61, 62, 63, 64, 71, 72, 78, 81, 85) seems to be the best bacterial detoxifier, perhaps because of its purity (64). It is claimed that all soaps detoxify (22). Sodium butyrate (121), sodium benzoate (120), sodium cholate (86), sodium dehydrocholate (125, 125a, 125b), sodium guttate (115), sodium hippurate (120), sodium mucate (120, 121), sodium oleate (83, 84, 117, 118), sodium oleomargarate (112), sodium palmitate (115, 116, 117, 118), sodium pyruvate (121) and sodium taurocholate (112) are some of the soaps which have been found to be detoxifiers.

It is generally accepted that castor oil soap or its purified form, sodium ricinoleate, is the most rapid detoxifier. The other detoxifiers require many minutes or hours to render toxins impotent, whereas ricinoleate seldom requires more than a few minutes. It is interesting to note that the best detoxifiers are sodium salts of saturated and unsaturated compounds. Vincent (120) found that sodium salts of the

lowest acyclic acids have no detoxifying power. Cryptotoxic action starts with sodium butyrate, $C_5$ is weakly cryptotoxic, $C_6$ is strongly, $C_7$ is weakly, $C_8$ is strongly active but is less so than $C_6$, $C_9$ is very feeble, $C_{10}$ is very active, $C_{11}$ acids are very cryptotoxic, $C_{12}$, $C_{14}$, $C_{15}$, $C_{18}$ are feebly cryptotoxic. Only sodium palmitate of the higher members of the series is cryptotoxic, .000002 mg. neutralize one lethal dose of tetanus toxin. The sodium salts of saturated acids of the acyclic series Vincent finds have very uneven cryptotoxic ability. The length of the chain is therefore not the only factor.

Vincent found that there is no absolute relationship between chemical structure, surface tension, and cryptotoxic power, and that salts of unsaturated acids are more active than salts of saturated acids. In this paper we report on the powers of methyl, trienthanolamine, and ephedrine-ricinoleate as detoxifiers.

Kozlowsky reported (49) that sodium ricinoleate destroyed the connective tissues, muscles, blood vessels, and blood of laboratory animals when injected in 2% aqueous solution. These results are sufficiently explained by Larson (53) who claims that Kozlowsky's results were entirely due to the fact that his sodium ricinoleate was prepared through barium. This element is very irritating to the tissues of the body.

## Value of Detoxification.

Five doses of toxin, each dose one week apart, have been given in scarlet fever prophylaxis. After two months 95% of those receiving these injections were reported as Dick negative. One injection of ricinoleated toxin has been reported to produce 95% Dick negatives in three weeks (61). The latter procedure is the more simple and more quickly effective. From several sources we have been informed, however, that the effectiveness of the ricinoleate method is not as great as was first indicated. The detoxification method has a great advantage over the old antitoxin method in that it avoids the use of foreign serum proteins and therefore avoids anaphylaxis.

The bite of venomous snakes is usually mortal. *In vivo* injections of sodium ricinoleate have been found (16) to detoxify snake venoms. This is a fertile field for further investigation.

Space will not permit other than mention of Besredka's article (9) on local immunity. Bacteriologists should have read it long before now. If his theory is sound, and it apparently is, detoxification shows promise as a means of treating skin infections. It was with this in

mind that the investigation with regard to staphylococcus aureus reported in this paper, was undertaken.

### Chemistry of Sodium Ricinoleate.

Sodium ricinoleate is the sodium salt of ricinoleic acid. Castor oil is a tri-glyceride of ricinoleic acid and is therefore the ready source of sodium ricinoleate. Castor oil is readily extracted from pulverized castor bean. In obtaining this oil it is important that one guard against the extremely poisonous protein, ricin, contained in the shells.

For use as a detoxifier, sodium ricinoleate must be very pure. Smallest amounts of impurities will completely destroy its detoxifying powers. Oleic, stearic and hydrostearic acids which are normally present in castor oil must be removed. Phenol, resorcinol and alkaline earth metals in small amounts completely destroy this property. Nonsol or pyrex glassware should be used in preparing the ricinoleate and for keeping it in storage. This glassware should be thoroughly cleansed with soap and rinsed several times with distilled water.

### Purpose of the Preparation of the Other Ricinoleates.

Sodium ricinoleate is the only ricinoleate which has been tested for its detoxifying powers. If detoxification is not dependent on the cation, it should be possible to detoxify with other ricinoleates than sodium. To prove that such a thing is possible three other ricinoleates were prepared. Methyl ricinoleate was prepared because it represents the most simple organic ricinoleate ester. Triethanolamine ricinoleate is a compound whose cation alone possesses marked surface tension lowering properties. It was hoped that when triethanolamine was chemically combined with the ricinoleate molecule a compound would result which would possess exceptional detoxifying ability. Ephedrine is noted for its bland effect on the mucous membrane. Combination of ephedrine with ricinoleate might result in a detoxifier which is less irritating than sodium ricinoleate and yet equally as effective. From the results we obtained, it is not possible to answer this question but there are indications that ephedrine ricinoleate has these properties.

### Detoxification of S. Aureus.

In the literature there is no report on the effect of ricinoleate on S. aureus. If Besredka's theory of local immunization has any value whatsoever and if ricinoleate will detoxify this ordinary pus former,

extensive application of the detoxification principle can be made in the treatment of acne and other skin disturbances. The only drawback in such an application would be in its irritating effect on the skin, but this would perhaps be avoided by dilution or mixing with other substances.

### Preparation of Sodium Ricinoleate.

At the time this work was done the method of Rider (94) was unpublished. Dr. W. P. Larson has kindly furnished us with a procedure for preparing a very pure sodium ricinoleate for detoxification purposes. We publish it here with his permission.

> Saponify cold press castor oil with c. p. sodium hydroxide and isopropyl alcohol in a reflux. Mix with several volumes of distilled water. Treat with hydrochloric acid to free of fatty acids, siphon off the water and place the fatty acids in two volumes of isopropyl alcohol. Stand at −20° C. for one week. Filter off the precipitated fatty acids on a Buchner filter. Saponify with c. p. sodium hydroxide. Pour into large evaporating dish and cut soap formed into small pieces. Let the isopropyl evaporate off and grind to a meal.

### Preparation of Methyl Ricinoleate.

Methyl ricinoleate is prepared by mixing 100 grams of purified ricinoleic acid, 1350 grams absolute methyl alcohol and 150 grams concentrated sulphuric acid and boiling under reflux condensers for 6½ hours. When cool, saturated salt solution is added. Methyl ricinoleate separates completely over-night. Remove the ricinoleate, wash with water until free from chlorides and sulphates and dry with calcium chloride.

### Preparation of Triethanolamine Ricinoleate.

Triethanolamine ricinoleate is prepared by saponifying cold press alcohol with c. p. sodium hydroxide in isopropyl alcohol under a reflux condenser. Mix with several volumes of distilled water and treat with hydrochloric acid to free of fatty acids. Siphon off the water and complete the separation in a separatory funnel. The fatty acids are then placed in two volumes of isopropyl alcohol and allowed to stand at −20° C. for one week. This precipitates out the saturated fatty acids. The product is then filtered on a Buchner filter. The filtrate is a solution of pure ricinoleate acid in isopropyl alcohol. One gram

of this solution is equivalent to 7.27 cc. sulphuric acid (normal). One gram of triethanolamine requires, then, 7.27/1.176 grams of ricinoleic acid solution to be exactly neutralized. In this preparation 50 grams of triethanolamine were heated on a steam bath for two hours with 309.1 grams of ricinoleic acid solution and the isopropyl alcohol evaporated. The triethanolamine ricinoleate prepared was 99.29% pure.

### Preparation of Ephedrine Ricinoleate.

Ricinoleic acid is prepared as in the first part of the triethanolamine ricinoleate procedure. To excess of this the alkaloid is added, heated on a steam bath and then the isopropyl alcohol evaporated off.

### Densities and Surface Tensions of Aqueous Solutions of the Ricinoleates.

| | Density | Surface tension |
|---|---|---|
| Sodium ricinoleate (3%) | 1.0010 | 63.9 dynes per cm. |
| Triethanolamine ricinoleate (3%) | .9980 | 57.0 " " " |
| Ephedrine ricinoleate (2%) | | 40.0 " " " |
| Methyl ricinoleate (3%) | .9980 | 67.0 " " " |
| Water (distilled) | 1.0000 | 73.0 " " " |

Density and surface tension determinations were made under standard conditions, the latter with the use of a Traube stalagmometer.

### Detoxification of S. Aureus.

In this test 1 cc. portions of a suspension of S. aureus with the different ricinoleates or castor oil soap were injected subcutaneously into white rats (Wistar) which had been shaved at the site of injection, the back. The culture was obtained from a human case and had been grown on nutrient broth for five days with 24 hour transplants. Each cc. of the injected ricinoleate-aureus mixture was found to contain 5,000,000 bacteria. Dilutions were made of the ricinoleate so that the final solution contains an even percentage. Before dilution the ricinoleates were autoclaved to sterilize and then diluted with sterile distilled water. The material when injected was at 35° C. Separate tests were made to determine the extent of antisepsis from the ricinoleate. Though there was a slight drop in the count it was not significant.

TABLE VI.

| Solution injected | Animal No. | Tissue reaction | Pus formation |
|---|---|---|---|
| S. aureus only 5,000,000 per cc. | LA1 | | abscess |
| | LA2 | | abscess |
| | LA3 | | abscess |
| | LA4 | | abscess |
| | 210 | | abscess |
| | 211 | | abscess |
| | 212 | | abscess |
| | 213 | | abscess |
| | 214 | | abscess |
| | 215 | | abscess |
| 8% Castor oil soap | LB1 | slight | |
| | LB2 | vigorous | |
| | LB3 | vigorous | |
| | LB4 | slight | |
| 5% Sodium ricinoleate | LB1 | 0 | |
| | LB2 | 0 | |
| | LB3 | very slight | |
| | LB4 | 0 | |
| 3% Sodium ricinoleate | LB5 | 0 | |
| | LB6 | 0 | |
| | LB7 | 0 | |
| | LB8 | 0 | |
| 3% Triethanolamine ricinoleate | 222 | 0 | |
| | 223 | 0 | |
| | 224 | 0 | |
| | 225 | 0 | |
| 3% Methyl ricinoleate | 216 | 0 | |
| | 217 | very slight | |
| | 218 | vigorous | |
| | 219 | 0 | |
| | 220 | 0 | |
| | 221 | slight | |
| 8% Castor oil soap and aureus | LC5 | vigorous | abscess |
| | LC6 | vigorous | 0 |
| | LC7 | vigorous | abscess |
| | LC8 | vigorous | 0 |

TABLE VI—cont.

| Solution injected | Animal No. | Tissue reaction | Pus formation |
|---|---|---|---|
| 5% Sodium ricinoleate and aureus | LD1 | sores | 0 |
| | LD2 | sores | 0 |
| | LD3 | 0 | 0 |
| | LD4 | 0 | 0 |
| 3% Sodium ricinoleate and aureus | LD5 | 0 | 0 |
| | LD6 | 0 | 0 |
| | LD7 | 0 | 0 |
| | LD8 | 0 | 0 |
| | 246 | 0 | 0 |
| | 247 | 0 | 0 |
| | 248 | 0 | 0 |
| | 249 | 0 | 0 |
| | 250 | 0 | 0 |
| | 251 | 0 | 0 |
| 3% Triethanolamine ricinoleate and aureus | 234 | 0 | 0 |
| | 235 | 0 | 0 |
| | 236 | 0 | 0 |
| | 237 | 0 | slight |
| | 238 | 0 | 0 |
| | 239 | 0 | 0 |
| 3% Methyl ricinoleate and aureus | 240 | slight | abscess |
| | 241 | slight | abscess |
| | 242 | 0 | 0 |
| | 243 | slight | 0 |
| | 244 | slight | 0 |
| | 245 | vigorous | abscess |

*Discussion.* The culture used was demonstrated to be virulent enough to cause abscesses. The concentration of castor oil soap used was too high to prevent irritation but too low to detoxify. Both castor oil soap and methyl ricinoleate are poor detoxifiers. Sodium and triethanolamine ricinoleates seem to be of equal detoxifying strength. This would indicate that the ricinoleate part of the molecule is a factor in detoxification (note that all solutions detoxified) but is not the only factor (note the difference in detoxifying power). Detoxification is certainly not due to surface tension alone. Staphy-

lococcus aureus can be detoxified by ricinoleate compounds to an extent sufficient to prevent pus formation.

### Detoxification of the Tubercle Bacillus.

The tubercle bacillus produces no true toxins but the bodies of the bacteria themselves cause necrosis of the tissue with subsequent caseation and abscess. Soon after the introduction of the bacilli into the tissues the surrounding tissues show irritation. The connective tissue cells swell and undergo mitotic division and are distinguishable by their large size and pale nuclei. Young guinea pigs are very susceptible to tubercle bacillus injections. When inoculated with a minute quantity of living bacilli they succumb to the disease. Intraperitoneal injection most rapidly produces infection. Death follows the injection of larger doses in ten to twenty days. The omentum clumps of sausage masses contain many bacilli. Serous fluid is to be found in both pleural sacs, the spleen enlarges and becomes covered with tubercles as is also the case with the liver and intestine. The tissues thicken around the site of inoculation. Neighboring lymph nodes become swollen. The mediastinal glands fill with cheesy material rich in organisms.

In the following test, the guinea pigs were individually caged and fed a diet of carrots, cabbage, bran and oats. Each animal weighed about 15 ounces at the commencement of the test. The tuberculosis culture used was that supplied by the Albany Medical College, it having been found the most virulent of a number of cultures tested. Inoculations were made in the animal's groin. The solutions injected were prepared in a manner identical to that in the previous test.

*Discussion.* Sodium ricinoleate protected two out of eight of the animals, triethanolamine ricinoleate and ephedrine ricinoleate each protected three out of eight animals. This might indicate that sodium ricinoleate is the poorest detoxifier of tubercle bacillus.

It is evident that the concentrations used are too small to completely protect the animals. This test does show, however, that the tubercle bacillus is "detoxified" to some extent and that detoxification should be investigated clinically with the expectancy of success comparable with that of Kline (47) and Morris and Dorst (81) (81a).

These results show detoxification is not due to any one single cation. Detoxification seems to be due to several or all of the following factors: (1) surface tension, (2) solubility of the detoxifier, (3) length of time of contact of detoxifier with toxin, (4) temperature of the

TABLE VII.

| Solution injected | Animal no. | Effect on intestine | Effect on spleen | Effect on mediastinals | Lymph glands |
|---|---|---|---|---|---|
| 3% sodium ricinoleate | 505 | none | none | none | none |
| | 506 | none | none | none | none |
| 3% Triethanolamine ricinoleate | 503 | none | none | none | none |
| | 504 | none | none | none | none |
| 2% Ephedrine ricinoleate | 507 | none | none | none | none |
| | 508 | none | none | none | none |
| Tubercle bacillus 3,000,000 per cc. | 500 | ulcered | ulcered | cheesy | enlarged |
| | 501 | ulcered | spotted | cheesy | enlarged |
| | 502 (died) | ulcered | spotted | cheesy | enlarged |
| 3% Sodium ricinoleate & T. B. | 470 | normal | spotted | enlarged | enlarged |
| | 471 | normal | spotted | cheesy | enlarged |
| | 472 | normal | normal | normal | normal |
| | 473 | normal | spotted | enlarged | cheesy |
| | 474 | ulcered | spotted | cheesy | cheesy |
| | 475 | normal | normal | normal | normal |
| | 476 | normal | enlarged | normal | cheesy |
| | 477 | normal | enlarged | normal | cheesy |
| 3% Triethanolamine ric. & T. B. | 480 | normal | cheesy | enlarged | cheesy |
| | 481 | normal | normal | normal | normal |
| | 482 | normal | normal | normal | normal |
| | 483 | normal | spotted | enlarged | cheesy |
| | 484 | normal | normal | normal | normal |
| | 485 | normal | spotted | enlarged | cheesy |
| | 486 | normal | spotted | cheesy | cheesy |
| | 487 | normal | spotted | normal | cheesy |
| 2% Ephedrine ricinoleate and T. B. | 490 | normal | normal | normal | normal |
| | 491 | normal | cheesy | cheesy | enlarged |
| | 492 | normal | enlarged | normal | enlarged |
| | 493 | normal | normal | normal | normal |
| | 494 | normal | spotted | normal | cheesy |
| | 495 | normal | spotted | cheesy | cheesy |
| | 496 | normal | normal | normal | normal |
| | 497 | normal | spotted | enlarged | cheesy |

mixture, (5) chemistry of the anion or cation (i. e., number of carbon atoms, unsaturated linkages, etc., (6) extent of ionization of the detoxifier, (7) the nature of the organism or toxin being acted upon and (8) adsorption. The presence of alkali earth metals interferes with detoxification.

## Summary and Conclusions

The theoretical and historical aspects of bacterial detoxification have been summarized.

The authors adhere to the view that detoxification is due not alone to surface tension depressant action of the detoxifier, nor solely to any particular anion or cation, but to the configuration of the molecule as a whole.

S. aureus in a suspension of 5,000,000 organisms per cc. of 3% sodium or tritehanolamine ricinoleate is detoxified sufficiently to prevent abscess formation when injected subcutaneously into albino rats. Methyl ricinoleate was not effective.

Tubercle bacilli in a suspension of 3,000,000 organisms per cc. of 3% sodium, 3% triethanolamine, or 2% ephedrine ricinoleate gave evidence of "detoxification" by the ricinoleates and the protection of some of the animals. The cases are too few to warrant a statistical expression of results.

The protective action against the bacteria tested was in no case due to germicidal action of the detoxifiers.

Further study of bacterial detoxification from a theoretical standpoint, and the development of therapeutic measures founded upon the present state of knowledge concerning this phenomenon are definitely worth while.

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